8-2007

Boiling and Microwaving Effects on Hydrophilic Oxygen Radical Absorbance Capacity of Frozen Vegetables

Merry Frances Rogers
University of Tennessee - Knoxville

To the Graduate Council:

I am submitting herewith a thesis written by Merry Frances Rogers entitled "Boiling and Microwaving Effects on Hydrophilic Oxygen Radical Absorbance Capacity of Frozen Vegetables." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Food Science and Technology.

John Robert Mount, Major Professor

We have read this thesis and recommend its acceptance:

Svetlana Zivanovic, William C. Morris

Accepted for the Council:
Carolyn R. Hodges

Vice Provost and Dean of the Graduate School

(Original signatures are on file with official student records.)

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A thesis
Presented for the Masters of Science
Degree
The University of Tennessee, Knoxville

Merry Frances Rogers

August, 2007

Dedication

This thesis is lovingly dedicated to my sweet Granma and precious Tookey.

Acknowledgements

I would like to thank Dr. Mount for his help and patience. I was exceptionally lucky he was willing to work with me, especially considering my only knowledge regarding food upon entering this program was that I enjoyed eating it. Dr. Mount is a true scholar, researcher, and teacher. I consider myself fortunate he was my major professor, and I will always remember him fondly.

I would also like to thank Dr. Zivanovic for her guidance and support. Her food chemistry class was one of my first food science classes, and I will always be impressed by her concern for her students.

I am also thankful for the assistance Dr. Morris provided. His assistance helped me receive the Westcott Scholarship award. The award truly aided in easing the financial strain experienced by leaving the work force to enter graduate school. Of course, learning about wine and the wine industry in Tennessee was fun as well.

Additionally, I would like to thank Kevin for his continued support throughout my time in graduate school. I especially appreciate his help during my transition from work to school. He always knew when to make me laugh and when to let me study.

I am forever grateful for my parents. They have always supported me and encouraged me to excel in academic pursuits. I am thankful they supported my return to school, especially since the return took me down a different path than I previously traveled. I also appreciate their financial assistance during this time and when I was an undergraduate student.

I would also like to thank my grandmother for her continued support, kindness, and patience. She is so precious to me. I love her dearly.

Of course, Tookey and Gretchen are thanked for their unconditional love and ability to make me laugh and smile.

Abstract

Decreased risks of chronic illnesses, such as cancer, occur with increased consumption of dietary antioxidants. Vegetables are a particularly rich source of dietary antioxidants but these are primarily water soluble compounds. This research determined effects of microwaving or boiling on the antioxidant capacities of commercially frozen vegetables. Hydrophilic components were extracted by Acetone/Deionized water/Acetic Acid (700:295:50, v/v) from commercially frozen broccoli, carrots, sweet corn, and sweet peas before and after microwaving for 5 min or boiling for 10 min. The Oxygen Radical Absorbance Capacity (ORAC) assay was employed to determine the antioxidant capacity. Additionally, color and texture analyses were performed.

ORAC values from uncooked, microwaved or boiled broccoli were 11.33, 8.04 and 5.72 μmol TE/g; ORAC values for peas were 10.2, 5.14 and 2.43 μmol TE/g; ORAC values for corn were 6.32, 8.12 and 4.45 μmol TE/g; and ORAC values for carrots were 2.95, 4.00, and 2.39 μmol TE/g.

No significant ORAC and texture correlations were determined. The only significant color and ORAC correlation was for the a^* value of peas ($p<0.05$). A negative moderate correlation existed; therefore, greener peas had greater ORAC values.

These results demonstrate that boiling vegetables for 10 min results in lower ORAC values and boiled broccoli, peas and corn contained significantly lower values ($p<0.05$) than uncooked broccoli or peas and microwaved corn. Boiled vegetables have been found to contain significantly lower water soluble nutrients due to loss into the cooking water. ORAC analysis of cooking water from each of the four vegetables

verified the loss of antioxidant constituents since the water was found to contain increased antioxidant capacity. The greatest ORAC values were found in the water after boiling broccoli and the lowest ORAC values were found in the water after cooking carrots. Addition of antioxidant capacities of cooked vegetables in nutritional databases would be useful to consumers wanting to increase consumption of antioxidants.

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1. Introduction

A growing body of scientific evidence supports the inverse relationship between increased consumption of dietary antioxidants and decreased chronic illnesses, such as cancer (Bengtsson and others 2006; Eberhardt 2005; Wu and others 2004). Dietary antioxidants are found in foods such as fruits, vegetables, cereals, legumes and other foods. Vegetables are a particularly rich source of antioxidants (Kurilich and others 2002). The primary compounds containing antioxidant properties in foods include carotenoids, fat-soluble vitamins (such as tocopherol), water-soluble vitamins (such as Vitamin C), and many phenolic compounds (Kalt 2005; Wu and others 2004).

Frozen vegetable consumption, other than potatoes, has averaged between 44 to 46.2 kg per person annually in the United States for the past 20 years. Broccoli, carrots, corn, and peas are among the most commonly consumed frozen vegetables in the United States (USDA 2005). Americans consumed 1.23 kg of broccoli, 0.64 kg of carrots, 3.95 kg of sweet corn, and 0.77 kg of sweet peas per person during 2005 according to USDA data.

The edible portion of these vegetables represents varying parts of each vegetable. For example, the root of the carrots, the flower of the broccoli, and the seeds of the corn and peas are consumed. These vegetables possess documented amounts of antioxidants (Wu and others 2004; Scott and Eldridge 2005; Hunter and Fletcher 2002). Frozen vegetables are typically cooked prior to consumption which impacts the antioxidant amount, particularly the amount of hydrophilic antioxidants, present following cooking (Wu and others 2004). The method of cooking may influence the amount of antioxidants lost from the frozen product.

The Oxygen Radical Absorbance Capacity (ORAC) assay is a popular method to determine the amount of antioxidants present in foods. The assay involves a hydrogen atom transfer (Huang and others 2005), and it measures the antioxidant capacity within a sample to provide protection against a pro-oxidant.

The objective of this research was to determine the effects of heating on antioxidant content in commercially frozen broccoli, carrots, sweet corn, and sweet peas. The vegetables were heated by either microwaving in a covered container or boiling in water. The changes in quality of the vegetables following the heating procedures were also determined by measuring color, pH, and texture attributes.

2. Literature Review

2.0.1 Antioxidants

Antioxidants are defined by a compound's ability to inhibit an oxidative reaction (Fennema 1996). Oxidative reactions occur in foods and within human bodies. In foods, these reactions often lead to premature senescence and degradation of nutritional value and flavor compounds. Oxidative reactions occur within foods due to reactive oxygen species (ROS) resulting from normal cell aerobic respiration (Ou and others 2002). Inherently humans' immune systems provide some protection against ROS; however, the immune system is not completely effective against ROS (Stratil and others 2006). Oxidative stress occurs when a disproportionate amount of ROS relative to the body's antioxidant defense are present. Consumption of foods rich in dietary antioxidants, which aid in inhibiting harmful oxidative reactions, is desirable for human health. Antioxidants inhibit oxidative reactions by various mechanisms (Fennema 1996). Antioxidants are capable of acting as reductants. Reductants are compounds that gain an electron with a possible loss of oxygen and/or hydrogen (Gutteridge and Halliwell 1994). Dietary antioxidants are capable of preventing the occurrence of some oxidative reactions in some instances and terminating the formation of ROS in other cases (Huang and others 2005). Inhibition of oxidative reactions is possible by antioxidants preventing the initiation step of the free radical chain reaction. An example of inhibition is the ability of some compounds to bind with metal ions, such as zinc, to prevent oxidative damage due to the metal ions (Gutteridge and Halliwell 1994). Termination of the formation of ROS occurs when the propagation step of the reaction is interrupted, as seen below in the example from Predicting the Activity of Phenolic Antioxidants: Theoretical Method,

Analysis of Substituent Effects, and Application to Major Families of Antioxidants by Wright and others, 2001 where RH is the substrate, $R^{\bullet}$ is a free radical, $RO_2^{\bullet}$ is a reactive oxygen species, and ArOH is the antioxidant:

initiation	$RH \rightarrow R^{\bullet}$
propagation	$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}$
hydrogen atom exchange	$RO_2^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$
termination	$RO_2^{\bullet} + ArOH \rightarrow ROOH + ArO^{\bullet}$

The termination is considered effective when $ArO^{\bullet}$ is a relatively stable free radical that is able to react slowly with the substrate, RH, and rapidly with $RO_2^{\bullet}$ (Wright and others 2001). In this instance, the antioxidant acted as a reductant to terminate the free radical reaction at the propagation step by donating a hydrogen atom.

2.0.2 Antioxidant Measurement Methodology

The use of different assays by research groups can result in reporting varying amounts of antioxidants found in various food products. Each assay typically employs a different free radical to use as the standard. The ability of the antioxidants to react with the free radical affects the antioxidant capacity. The trolox equivalent antioxidant capacity (TEAC) assay uses the 2,2'-azinobis(3-ethylbenzothiazoline 6-sulfonate) ($ABTS^{+}$) radical cation (Bahorun and others 2004). This assay's antioxidant capacity amount is based from the antioxidant's ability to "scavenge the performed radical cation $ABTS^{+}$ relative to that of the standard antioxidant Trolox C" (Bahorun and others 2004). The ferric reducing antioxidant capacity (FRAP) assay does not employ a free radical. Instead this assay measures the ability of assumed antioxidants to reduce a Fe^{3+}/tripyridyl-*s*-triazine complex to its Fe^{2+} form (Bahorun and others 2004). A color

change occurs simultaneously with reduction at 593 nm, and the color change is correlated to the amount of assumed antioxidants present in the sample (Huang and others 2005). However, one limitation to the FRAP assay is that some compounds other than the Fe^{2+}/tripyridyl-*s*-triazine complex might cause interference due to their UV-Vis absorption at 593 nm (Ou and others 2002). One example of such a compound is bilirubin. Bilirubin, when oxidized, converts to beliverdin which has a strong absorption at 593 nm (Ou and others 2002). Additionally, some compounds possibly have significant antioxidant capacities but are not able to reduce the Fe^{3+}/tripyridyl-*s*-triazine complex, such as glutathione, ascorbic acid, quercetin, tannic acid, and thiol compounds (Prior and others 1999; Ou and others 2002).

2.1 Frozen Vegetables' Information

Broccoli, carrots, sweet corn and sweet peas were the four most consumed frozen vegetables over the past 10 years in the United States, not including potatoes (USDA 2005). These vegetables also are important sources of water soluble antioxidants as determined as hydrophilic ORAC contents (Wu and others 2004). Appendix E contains USDA nutrient data for the four frozen and frozen, boiled vegetables.

The quality of frozen vegetables is defined in the United States Standards for Grades with color and texture as two of the common quality attributes assigned to broccoli, carrots, corn and peas. The color of Grade A frozen broccoli should be "reasonably good", frozen carrots must possess a "good color where a good color is defined as a bright orange-yellow color", sweet corn must possess a "bright uniform color" and peas should be a "bright uniform green color". The color of the vegetables is important since many of the natural colorants are either precursors to antioxidants or

contain antioxidant capacity themselves. Texture of Grade A frozen broccoli, carrots, corn and peas should be a "tender texture". The texture in vegetables consumed by today's consumers should be maintained rather than cooked to a very soft final product. Microwaving for approximately 6-8 min per pound or boiling for approximately 3-10 min per pound are recommended on most commercial packages of these vegetables sold today.

2.1.1 Broccoli

Broccoli is purported to be a native plant of Italy (Economic Research Service/USDA 1999). It is a member of the Brassicaceae family and belongs to the species *Brassica oleracea* (Wildman 2001). The Brassicaceae family is more commonly known as the Cruciferae family. Broccoli grows best in cool climates; therefore, in the United States of America the majority of broccoli is grown in cool coastal areas of California generally during the winter and early spring (Economic Research Service/USDA 1999). The Calabrese variety and the Italian variety are the two predominate types of broccoli. Calabrese is more common in the United States of America, and the Italian variety is more common in Great Britain and other regions in Europe (Everett 1981). Broccoli did not become a largely consumed vegetable in the United States until the 1970's (Economic Research Service/USDA 1999).

Vegetables in the Brassicaceae family are suggested to provide better health benefits against cancer than many other vegetables according to recent epidemiological studies (Wildman 2001). The primary reason is due to the presence of glucosinolates in these vegetables. Glucosinolates are water-soluble plant compounds that are "relatively unique secondary metabolites of amino acids" (Pereira and others 2002; Wildman 2001).

Glucosinolates actually have no particular activity that is beneficial to human health; however, their byproducts which are formed as the vegetable is chopped or crushed do possess a bioactivity that is considered anticarcinogenic (Wildman 2001). The prevailing glucosinolate in broccoli is glucoraphanin, and its byproduct is sulforaphane. Sulforaphane acts as an antioxidant and is credited with possesssing atypical anticancer activity (Keck and others 2003).

Additionally, broccoli contains ascorbic acid, flavonoids, tocopherols and carotenoids (Kurilich and others 2002). The flower bud of broccoli contains the highest levels of antioxidants, followed by the floret stalks and then the main stem. Glycosides of flavonoids, conjugates of flavonoids, phenolic acids, conjugates of phenolic acids, and relatively minute amounts of anthocyanins are present in the flower buds of broccoli (Bengtsson and others 2006). Quercetin and kaempferol are the main glycosides and are present in significant amounts of the hydrophilic extract of broccoli flower buds (Bengtsson and others 2006; Kurilich and others 2002). Quercetin is particularly known as a very powerful flavonoid, and studies indicate it aids in preventing cancer, heart disease, respiratory disease, cataracts, and other health maladies (Lu and others 2006; Bahorun and others 2004).

The amount of antioxidants reported as present in uncooked broccoli varies significantly depending upon the assay employed and other factors. Unprocessed broccoli was found to possess 2.85 ± 0.56 μmol Trolox/g employing the TEAC assay and 3.36 ± 0.56 μmol Fe^{2+}/g employing the FRAP assay (Bahorun and others 2004). The ORAC assay found uncooked broccoli to contain 15.9 μmol Trolox equivalent/g (Wu and others 2004). Additional possible explanations for the varying broccoli antioxidant

amounts are the varying geographical locations and cultivars. Different extraction methods were employed which might further explain the inconsistent reported amounts of antioxidants in broccoli.

2.1.2 Carrots

Carrots belong to the *Umbelliferae* family and presumably originated in Asia and the Near East (Ensminger and others 1994). The root is the edible portion of the vegetable, and initially carrot roots had purplish hues. However, European agriculturists selectively bred carrots containing increased amounts of carotene to produce orange-colored carrots in the 17th century. The orange-colored carrots were introduced to North America as the colonies were settled. Carrots used for processing purposes are grown primarily in Washington, California, and Wisconsin (Brunke 2006). Temperatures ranging from 15.6^{o}C to 21.1^{o}C at the end of the growing season provide an ideal climate for optimum growth of carrots (Ensminger and others 1994).

Carrots are considered a functional food and are known to contain phenolics, carotenoids, and polyacetylenes (Hager and Howard 2006; Howard and Dewi 1996). Carrots possess a relatively small amount of phenolics compared to many other vegetables; therefore, their antioxidant capacity is normally lower than these vegetables (Hager and Howard 2006; Ou and others 2002; Wu and others 2004). The periderm tissue in carrots contains the greatest amount of phenolics; however, phenolics are ubiquitous in the root (Hager and Howard 2006). On a cellular level phenolics are generally located in the vacuole and apoplast (Kalt 2005). Phenolics are recognized as excellent reductants due to their chemical structure. The basic phenolic structure is that of a six-member aromatic ring with a hydroxyl group directly bonded to it (Fennema

1996). This structure allows for stabilization of free radicals and termination of the propagation step (Hager and Howard 2006). Thus, phenolics are readily capable of preventing further formation of ROS, and they are considered excellent antioxidants.

Carotenoids are another powerful antioxidant present in carrots. The phloem tissue of the carrots has the greatest amount of carotenoids (Hager and Howard 2006; Howard and Dewi 1996). The carrot peel has intermediate levels of carotenoids, and the xylem tissue has the least amount of carotenoids (Hager and Howard 2006; Howard and Dewi 1996). The cellular location of carotenoids is normally in the chromoplast and the chloroplast (Kalt 2005). Carotenoids are long hydrocarbon chains with conjugated double bonds frequently terminated with a ring structure at either or each end. The conjugated double bonds allow for stabilization of an electron from a ROS (Hager and Howard 2006). This stabilization allows for carotenoids to possess antioxidant capacity.

2.1.3 Sweet Corn

Corn is native to the United States. Corn is a member of the *Poaceae* family, the *zea mays* genus, and the subspecies *mays* (Ensminger and others 1994). Therefore, its scientific classification is *Z. mays* ssp. *mays.* The most common varieties of corn today are dent corn and sweet corn. It is commonly grown in the Midwest, particularly in Iowa, Illinois, Nebraska, Minnesota, and Indiana (Ensminger and others 1994). Corn grows particularly well in generously fertilized, damp areas that experience warm nights (Ensminger and others 1994).

Sweet corn, in particular, is used for human consumption (Ensminger and others 1994). Corn is considered a very good source of Vitamin C and various carotenoids (Ensminger and others 1994; Scott and Eldridge 2005). Depending upon the variety of

corn, for example corn that is more yellow in color, it may also be a significant source of Vitamin A. In one study examining the White Shoepeg cultivar and the Golden Whole Kernel cultivar common carotenoids present were lutein, zeaxanthin, α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene (Scott and Eldridge 2005). Lutein and zeaxanthin were the predominate carotenoids present in both cultivars accounting for approximately 70% or more of the total carotenoids. Lutein and zeaxanthin, as carotenoids possessing antioxidant activity, are capable of quenching ROS (Fennema 1996). Therefore, health-related benefits may be obtained from consuming foods rich in these carotenoids, such as corn. In particular, an increased level of lutein in the diet is purported to minimize the risk of age-related macular degeneration (Richer and others 2004).

2.1.4 Sweet Peas

Pisum sativum, which is commonly known as the garden pea, is a legume that probably originated in Europe and Central Asia (Ensminger and others 1994). Peas do not tolerate hot conditions very well; therefore, the majority of peas grown in the United States are produced in Northern states (Ensminger and others 1994). Cultivation of peas to possess valuable characteristics occurred during the 19th Century. In the early 1800's peas were brought to America. Peas are still a part of the American diet and contain many antioxidants. Ascorbic acid, thiamine, riboflavin, β-carotene, α-tocopherol, quercetin, pyrrolylnorleucine and other polyphenols are antioxidants in peas (Nilsson and others 2004). The water-soluble antioxidants contribute more significantly to the total amount of antioxidants in peas than the water-insoluble antioxidants, and ascorbic acid is

the most prominent antioxidant present in peas (Nilsson and others 2004). Peas have powerful antioxidants capacities due to the combination of ascorbic acid, carotenoids, and phenolic compounds present in them.

2.2 Processing Effects on Antioxidants

Antioxidants are generally susceptible to processing effects, including home preparation methods. Microwaving and boiling are the most common methods used by consumers to cook vegetables at home. Broccoli, carrots, corn and peas experienced varying changes in their antioxidant capacity due to microwaving and boiling in water.

2.2.1 Broccoli

The majority of studies examining antioxidant changes in broccoli upon microwaving or boiling suggest a decrease in antioxidant activity occurs upon cooking. As stated previously, the main glycosides with antioxidant capacity in broccoli florets are quercetin and kaempferol. Isoquercitrin, kaempferol 3-*O*-glucoside and kaempferol diglucoside are additional minor glucosides present in broccoli (Price and others 1997).

Wu and coworkers (2004) found that uncooked broccoli contains 15.9 μmol Trolox equivalent/g when assayed used ORAC and cooked broccoli decreased in total antioxidant capacity to a value of 12.59 μmol TE/g. In a study by Price and others (1997) broccoli florets were boiled in water for 15 min and analyzed. The data show a significant loss of antioxidants occurred with only 18% of the initial antioxidants remaining in the broccoli upon cooking (Price and others 1997). The antioxidant loss was reported as most likely due to the large surface area of broccoli allowing for leaching

of the antioxidants into the water during the prolonged cooking time (Price and others 1997).

A study by Zhang and Hamauzu (2004) also supports the research by Price and others. In their study the antioxidant activity of broccoli florets and stems decreased gradually during microwaving and boiling (Zhang and Hamauzu 2004). Only 35% and 34.7% of the antioxidant activity in the broccoli florets and stems respectively was retained after boiling in water for five min (Zhang and Hamauzu 2004). A similar trend was exhibited by microwaving the broccoli florets and stems for the same amount of time (Zhang and Hamauzu 2004). Additionally total carotenoids, especially β-carotene, decreased during both methods of cooking (Zhang and Hamauzu 2004). However, it is noteworthy that the carotenoid lutein increased by 26.7% upon cooking for 5 min (Zhang and Hamauzu 2004). Ascorbic acid also considerably decreased during microwaving and boiling in water (Zhang and Hamauzu 2004). It is noteworthy that the process used to microwave the vegetables in the study by Zhang and Hamauzu (2004) was essentially the same as boiling. The broccoli was cooked in a microwave by placing the broccoli in 200 mL of boiled water and then cooking in the microwave (Zhang and Hamauzu 2004). Therefore, the broccoli was essentially boiled, so it is expected that their data from boiling and microwaving should agree. Contrary to the previous research, one study found that boiling and microwaving broccoli increased the antioxidant activity. This research found an increase in antioxidant activity of 15.90% after boiling broccoli for five min and an increase of 16.68% upon microwaving for 1 1/2 min (Turkmen and others 2006). The increase in antioxidant activity following cooking is possibly due to the inactivation of peroxidases at high temperatures (Turkmen and others 2006; Gazzani and

others 1998). An additional possibility is that reactions occur due to the high temperatures experienced during cooking that produce products possessing antioxidant activity (Turkmen and others 2006).

2.2.2 Carrots

The total antioxidant capacity of frozen carrots also altered with cooking. The major antioxidants present in carrots are phenolics and carotenoids. Phenolics are present throughout the carrot, and carotenoids are mainly located in the carrot root tissues (Hager and Howard 2006). The chemical structure of the antioxidants impacted the antioxidant changes during processing (Hager and Howard 2006). The solubility of antioxidants was determined by their chemical structure. Many phenolics' chemical structures are polar; therefore, these antioxidants were more susceptible to thermal processing with an aqueous medium than non-polar antioxidants (Hager and Howard 2006). Thus, phenolics were more readily lost by microwaving and boiling in water than other antioxidants, such as carotenoids. Carotenoids are non-polar in nature; therefore, they were more resistant to microwaving and boiling in water (Hager and Howard 2006).

The impact upon antioxidants with thermal processing was also a function of the antioxidants location within the plant (Hager and Howard 2006). Cell membrane degradation occurred with thermal processing; therefore, antioxidants, such as some phenolics, located in the cell membrane were lost (Hager and Howard 2006). In contrast, other phenolics attached to the cell wall became more available following thermal processing (Hager and Howard 2006). Carotenoids were increased upon cooking due to "tissue softening and destruction of the membrane-protein complex" (Hager and Howard 2006). Additionally, thermal processing resulted in the inactivation of carotene oxidizing

enzymes which also allowed for an increased presence of carotenoids following processing (Hager and Howard 2006; de Sá and Rodriguez-Amaya 2003).

Uncooked carrots were found to have a total antioxidant capacity of 12.15 μmol TE/g (Wu and others 2004). This amount decreased upon boiling the carrots to 3.71 μmol TE/g (Wu and others 2004). The TEAC assay and FRAP assay determined the amount of antioxidants in uncooked carrots to be 0.43 ± 0.01 μmol TE/g and 0.60 ± 0.01 Fe^{2+}/g respectively (Bahorun and others 2004). Frozen carrots were found to contain 885 ascorbate equivalents nmol/g (Hunter and Fletcher 2002). The varying total antioxidant capacities in carrots are possibly explained by the aforementioned reasons discussed with broccoli.

2.2.3 Corn

Cooking frozen corn provided varying outcomes regarding antioxidant capacity. As previously mentioned, carotenoids are the main antioxidants present in corn. More specifically, the primary antioxidants in corn are lutein and zeaxanthin (Scott and Eldridge 2005). The impact of thermal processing on the antioxidant capacity of White Shoepeg corn and Golden Whole Kernel corn was examined by canning corn in a study by Scott and Eldridge (2005). Prior to thermal processing the dominate carotenoid in White Shoepeg corn was zeaxanthin and the primary carotenoid in Golden Whole Kernel corn was lutein (Scott and Eldridge 2005). Additionally, α-cryptoxanthin, β-cryptoxanthin, α-carotene, and β-carotene were detected in the Golden Whole Kernel corn (Scott and Eldridge 2005). Statistically there were no differences in the amount of carotenoids present in the fresh and the canned corn with the exception of α-carotene

(Scott and Eldridge 2005). The amount of α-carotene in the canned Golden Whole Kernel corn decreased by 61.9% compared to the fresh Golden Whole Kernel corn (Scott and Eldridge 2005).

Uncooked corn had a reported total antioxidant capacity of 7.28 μmol TE/g (Wu and others 2004). Frozen corn had a total antioxidant capacity of 5.22 μmol TE/g (Wu and others 2004). The reported total antioxidant capacity of canned corn was 4.13 μmol TE/g (Wu and others 2004). Additional research found uncooked corn having 8.3 ± 0.03 μmol vitamin C equivalents/g (Dewanto and others 2002).

Lutein and zeaxanthin are oxygenated carotenoids; therefore, they are considered xanthophylls. Xanthophylls are susceptible to heat, oxygen, pH, and light; however, canning minimized the affect of light, pH, and oxygen (Scott and Eldridge 2005). The canning temperature induced isomerization of lutein and zeaxanthin according to research by Schwartz and Updike (2003). Thermal processing of corn resulted in an increased total amount of lutein compared to the amount of lutein in fresh corn (Schwartz and Updike 2003). Lutein is known to be more thermally stable than hydrocarbon carotenoids; therefore, possibly the increase in lutein is a result of the less thermally stable hydrocarbon carotenoids leaching into the canning medium (Ogunlesi 1979; Scott and Eldridge 2005; Schwartz and Updike 2003; Weckel and others 1962). Thus, an explanation for the lack of a decrease in antioxidant capacity in canned corn is that a greater concentration of lutein is present following thermal processing (Schwartz and Updike 2003). Additionally, the possibilities exist that the inactivation of carotenoid-oxidizing enzymes occurred (Baloch and others 1977; Schwartz and Updike 2003) and/or disruption of carotenoids-protein complexes aided in the efficiency of carotenoids

extraction (Schwartz and Updike 2003; Kirk and others 1978).

2.2.4 Peas

Peas' antioxidant capacity often changed due to cooking. Peas possess relatively high levels of water-soluble and lipid-soluble micronutrients, such as ascorbic acid, β-carotene, thiamine, and riboflavin (Nilsson and others 2004). Some of the aforementioned micronutrients also have antioxidant capacity. In particular, ascorbic acid is a water-soluble antioxidant and β-carotene is a lipid-soluble antioxidant (Nilsson and others 2004). Ascorbic acid contributes a large portion of the total antioxidant activity of peas (Hunter and Fletcher 2002).

Uncooked peas had a reported total antioxidant activity of 1827 nmol ascorbate equivalents/g, and the total antioxidant activity of microwaved peas was 1867 nmol ascorbate equivalents/g (Hunter and Fletcher 2002). Peas boiled for 3 min possessed a total antioxidant activity of 1588 nmol ascorbate equivalents/g and those boiled for 8 min were found to have a total antioxidant capacity of 1252 nmol ascorbate equivalents/g (Hunter and Fletcher 2002). Additional research found frozen peas and canned peas to have a total antioxidant activity of 6 μmol TE/g and 3.84 μmol TE/g respectively.

Turkmen and others (2005), employing the 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) assay, found the total antioxidant activity of frozen peas, when cooked by either microwaving, boiling or steaming, did not statistically change from the total antioxidant activity of the uncooked frozen peas. Research by Hunter and Fletcher (2002), employing the FRAP assay, examined the change in total antioxidant capacity by microwaving for 2 min, boiling for 3 min, or "overcooking" by boiling for 8 min. Interestingly, the amount of time the researchers consider "overcooking" is

approximately the amount of time many frozen food manufacturers suggest boiling frozen vegetables. It was determined that microwaving the peas resulted in no significant loss of water-soluble or lipid-soluble antioxidants (Hunter and Fletcher 2002). Boiling resulted in a slight loss of water-soluble and lipid-soluble antioxidants; however, a 39% loss of ascorbate occurred (Hunter and Fletcher 2002). This is counterintuitive since ascorbate is one of the predominate hydrophilic antioxidant present in peas. However, during cooking the amount of non-protein sulfydryl antioxidants increased; thus, negating some of the effects of the ascorbate loss (Hunter and Fletcher 2002). "Overcooking", which was boiling for 8 min, resulted in a 34% loss of total antioxidant activity and a 61% loss of ascorbate (Hunter and Fletcher 2002).

Additional research by Ewald and others (1999) determined some of the antioxidants present in peas were relatively heat stable. For example quercetin, a flavonoid, was regarded as being heat stable in boiling water. However, in the aforementioned research the peas were only boiled for 3 min. Had the peas experienced a longer treatment time a notable decrease in quercetin might have occurred.

3. Materials and Methods

3.1 Frozen Vegetables

Broccoli, carrots, sweet corn and sweet peas were each obtained from a single lot from a commercial frozen foods distributor. Photographic images are available for broccoli and carrots in Appendix G. Broccoli was either Legacy, Domador, or Monaco cultivars. Carrots were imported from Mexico and the variety was unknown. Sweet corn was the Syngenta GH2042 cultivar, and the sweet peas were the Early Freezer-680 cultivar. These vegetables were chosen because they were commonly consumed frozen vegetables (http://www.ers.usda.gov/data/foodconsumption/spreadsheets/vegfrz.xls#FarmPcc!A1, Accessed April 27, 2007). They represented a cross section of varying edible portions of plant (florets, roots or seeds). Previous studies indicated significant quantities of antioxidants in these vegetables (Wu and others 2004; Hunter and Fletcher 2002).

3.2 Heating Methods

The two methods of heating frozen vegetables typically recommended on the package are boiling and microwaving. These two methods were selected and the times were based on recommended cooking procedures by frozen food manufacturers.

3.2.1 Boiling

Two hundred gram samples of each vegetable were boiled in 200 mL of tap water. This amount was chosen as it was a commonly recommended serving size by frozen food manufacturers. This quantity of product provided enough sample for antioxidants analysis and other quality evaluations. Initially, 200 mL of tap water was

added to a 2 quart, 16 mm stainless steel pot on a 1200 W maximum burner of the electric Frigidaire Classic Series stovetop present in the pilot plant in the Food Science and Technology Department at The University of Tennessee. This amount of water covered the 200 g sample of each of the four vegetables; thus, providing a more uniform cooking medium. The stovetop was initially on the highest setting, which was Setting 6. Once the water boiled, 200 g of a selected vegetable were added and the lid was placed on the pot. The temperature was measured with thermocouples. Appendix I describes the protocol employed to use the thermocouples. Once the water reached a simmer, where the temperature was approximately 100^{o}C, the stovetop setting for the burner was changed to Setting 2. This setting was the minimum needed to maintain a simmer (approximately 97.8^{o}C – 101.9^{o}C). The container was removed from the heat 10 min after the 200 g of vegetable were added. The vegetables were transferred to a 1.75 quart Pyrex glass bowl.

Following cooking, the vegetables, liquid and 1.75 quart Pyrex bowl were weighed. The liquid was decanted from the vegetable using a strainer. The decanted liquid was placed into a Whirlpak bag which was then placed into a dark at -17.78^{o}C. The weight of the strainer was taken prior to and following straining broccoli to allow for determination of the broccoli florets remaining in the strainer. The vegetables and 1.75 quart Pyrex bowl were weighed. The vegetables were then divided approximately in half. One half of the vegetables were weighed, placed in a freezer bag, and frozen. The frozen vegetables were weighed and then freeze dried (Virtus, model number FFD-15-WS, Gardiner, NY) using a freeze dryer. The vegetables were weighed after freeze drying. The freeze-dried vegetables were used for ORAC analysis. The pre and post

freeze-drying weights allowed for dry weight to wet weight ORAC conversions.

The remaining portion of the cooked vegetables was cooled in an ice bath to approximately 25^{o}C. Texture and color analyses were performed on these vegetables within 2 h of cooling to room temperature. The vegetables were then frozen and stored for pH measurements.

3.2.2 Microwaving

The second cooking method examined was microwaving. In order to ensure comparable cooking in the microwave, initially water was heated for 5 min to increase the interior temperature of the microwave prior to the initial cooking. The elevated interior temperature remained fairly constant throughout cooking of the other vegetables. Two hundred grams of vegetables were placed into a 1.75 quart Pyrex glass bowl and 30 mL of tap water were added. A lid was set on top of the bowl, and the bowl was placed in the front center of an 1100 W Sharp microwave. The vegetables were cooked for 5 min on the high power setting and the bowl of vegetables was continuously rotating inside the microwave. Following the 5 min cooking time, the vegetables were equilibrated in the microwave for 1 min to ensure a more thorough cooking of the vegetables. The post-cooking process used for the boiled vegetables was repeated for the microwaved vegetables.

The cooked vegetables were separated into equal portions. A portion of each of the vegetables was immediately placed into a freezer at -18^{o}C in the dark. The other portion of the cooked vegetable sample was placed in an ice bath until it cooled to room temperature. These vegetables were then used for color analysis and texture analysis within 2 h. Upon completion of the analyses, the vegetables were frozen in a -18^{o}C

freezer and held for pH analyses.

3.3 Sample Preparation for Extraction

Approximately 100 g of each vegetable for each cooking method and for each of 5 replications were removed from the freezer, weighed and then freeze dried in batches in a freeze drier. The vegetables were freeze dried to improve extraction efficiency (Kurilich and others 2002; Ou and others 2002; Wu and others 2004). The freeze-dried vegetables were placed into freezer bags and stored at -18^{o}C in the dark. The freeze-dried vegetables were divided into two equal parts and one part from each of the 60 batches (4 vegetables, 3 heating treatments, 5 replications) was ground with a Wiley Mill Model (Thomas Scientific, model number 3383-L10, Swedesboro, NJ) using a size 20 mesh sieve. The ground vegetables were then vacuum packaged and stored in at -18^{o}C in the dark. The ground vegetables were analyzed for ORAC content within 30 d.

3.4 Oxygen Radical Absorbance Capacity Assay Preparation

3.4.1 Vegetable Sample Extraction for ORAC Assay

The vegetable samples were prepared for ORAC analysis by extraction with a solution of Acetone/Deionized water/Acetic Acid (AWA, 700:295:50). A 0.5 g freeze dried and ground vegetable sample and 5 g of sand were added to a 50 mL beaker. Twenty five milliliters of AWA were added to the beaker. The beaker was wrapped in Aluminum foil, covered with parafilm, and stirred on a Corning Stirrer/Hotplate on setting four with no heat for an h. The contents of the beaker, following stirring for one h, were filtered through a Calbiochem Miracloth (Lot number B63488, San Diego, CA) into a 25 mL volumetric flask. The filtrate was brought back to 25 mL with AWA. This

sample is then diluted with phosphate buffer to a 1:100 dilution. The diluted vegetable extract was used for the ORAC assay.

3.4.2 Phosphate Buffer Solution Preparation

The 75 mM phosphate buffer working solution was prepared from 75 mM monopotassium phosphate salt (Solution A) (Fisher Chemical, formula weight 136.09), 75 mM dipotassium phosphate salt (Solution B) (Fisher Chemical, formula weight 174.18), and deionized water.

Solution A was prepared by weighing 10.21 g of monopotassium phosphate salt and transferring the salt to a 1000 mL volumetric flask. Seven hundred milliliters of deionized water were added to the volumetric flask along with a magnetic stir bar. The volumetric flask was placed on a stir plate and stirring occurred until the monopotassium phosphate was dissolved. The stir bar was removed and deionized water was added to the flask until the 1000 mL mark was reached.

Solution B was derived from the dipotassium phosphate salt, and it was prepared by weighing and transferring 13.06 g of dipotassium phosphate to a 1000 mL volumetric flask. Seven hundred milliliters of deionized water were added to the volumetric flask along with a magnetic stir bar. The volumetric flask was placed on a stir plate and stirring occurred until the dipotassium phosphate was dissolved. The stir bar was removed and deionized water was added to the flask until the 1000 mL mark was reached.

The phosphate buffer working solution was prepared by pouring 800 mL of Solution B into a 1000 mL beaker and adding a magnetic stir bar. A pH electrode was inserted into the solution and the pH was determined. Approximately 150 mL of

Solution A were added to the beaker containing Solution B until a final solution pH of 7.4 was reached.

3.4.3 Trolox Standards Preparation

Trolox, scientifically known as 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, from Aldrich Chemicals, formula weight 250.29, was used to prepare a Trolox stock solution. Twenty five mg of Trolox were dissolved into 100 mL of phosphate buffer working solution to make a 1 mM Trolox solution. The 1 mM Trolox solution was then diluted to a 500 mM solution. This solution was transferred to 1.8 mL Eppendorf tubes via 1.3 mL aliquots and stored at -85^{o}C until use. Prior to an ORAC analysis, dilutions of the Trolox stock solution were performed. The Trolox stock solution was thawed and one milliliter was added to a 15 mL dilution tube along with 9 mL of the phosphate buffer working solution. The solution was vortexed to make a 50 microMolar Trolox working solution. Serial dilutions of the working solution were performed by adding 5 mL of the 50 μM Trolox working solution to 5 mL of phosphate buffer working solution. This solution was then vortexed; thus, making a 25 μM Trolox solution. The serial dilutions were repeated until a solution with a final molarity of 3.125 μM Trolox was achieved. The solutions of Trolox standards (25 μM Trolox, 12.5 μM Trolox, 6.25 μM Trolox, and 3.125 μM Trolox) were used with each ORAC assay.

3.4.4 Fluorescein Solution Preparation

Fluorescein disodium salt (Aldrich Chemicals, Milwaukee, Wisconsin), formula weight 376.28, was used to prepare a Fluorescein solution. Initially the solution was prepared by dissolving 0.0225 g of Fluorescein disodium salt into 50 mL of phosphate

buffer working solution and vortexing. This solution was then diluted by adding 250 µl into 50 mL of phosphate buffer working solution and vortexing. The diluted solution was transferred into 1.8 mL Eppendorf tubes via 1.3 mL aliquots and stored at -85^{o}C until use.

The Fluorescein working solution used in the ORAC assay was prepared by transferring 800 µL of Fluorescein solution removed from the freezer into 50 mL conical tube containing 50 mL of phosphate buffer working solution. The solution was then vortexed and stored in a 37^{o}C water bath approximately one h prior to use.

3.4.5 AAPH Solution Preparation

AAPH, scientifically known as 2,2'-Azobis(2-amidinopropane) dihydrochloride, from Wako Chemicals USA, Inc. (Richmond, VA) with a formula weight of 271.193, was used to make the AAPH solution. Initially, 5 mL of phosphate buffer working solution are incubated in a water bath at 37^{o}C until thoroughly heated. Following heating, 0.108 g of AAPH were dissolved into the 37^{o}C phosphate buffer working solution and vortexed.

3.4.6 Forty eight Well Microplate Preparations

Trolox standards (25 µM Trolox, 12.5 µM Trolox, 6.25 µM Trolox and 3.125 µM Trolox), phosphate buffer working solution, Fluorescein working solution, and vegetable sample extracts were placed into a 48 well microplate as seen in Figure 3 and Figure 4 of Appendix H. The vegetables extracted from the samples prepared during the first replication were assayed for ORAC content by comparing each heating method to the frozen vegetable sample on each plate (Figure 3). The vegetables prepared during

replications two through five were assayed with extracts from microwaved and boiled vegetable samples with the extract from the frozen vegetable control sample (Figure 4). One vegetable was used for each ORAC assay and the microplate contained uncooked vegetable sample extracts at a 1:100 dilution, microwaved vegetable sample extracts at a 1:100 dilution, and boiled vegetable sample extracts at a 1:100 dilution along with Trolox standard solutions, the fluorescein working solution, and the phosphate buffer working solution. This format minimized any inherent differences within the ORAC plate reader for a specific vegetable.

Additionally, following all ORAC assays with vegetable samples the retained water was analyzed using the ORAC assay as seen in Figure 5 and Figure 6 of Appendix H.

3.4.7 Operation of the BMG Fluostar optima plate reader

The prepared 48 well microplate was placed into the plate reader and incubated inside the plate reader at 37^{o}C for a minimum of 10 min. The test protocol included the following basic parameters: Costar 48 for the microplate, a Position delay of 0.3 s, 1 for the Number of kinetic windows, a measurement start time of 0.0 s, 15 for the number of flashes per cycle, 210 s for the cycle time, fluorescence intensity for the filters and integration, 1 for the number of multichromatics, and 485 nm and 520 nm for the excitation and emission filters respectively. The layout of the microplate as shown in Figure 3 or Figure 4 was entered depending upon the replication performed. Fluorescein working solution was placed into the container that fed pump 1 and the pump was set to deliver 200 µL at a pump speed of 420 µL/s. AAPH solution was placed into the container that fed pump 2 which was set to deliver 35 µL at 420 µL/s. Additionally, the

orbital shaking mode was set to a shaking width of 4 mm before each cycle for additional shaking, and a shaking time of 8 s.

3.5 Oxygen Radical Absorbance Capacity Assay Determinations

The area of the ORAC curves for the 23 phosphate buffer wells were analyzed and any area values that were more than plus or minus two standard deviations were removed as outliers. The average of the remaining areas was used to determine the background fluorescence and subtracted from the areas of the curves for the Trolox standards and vegetable samples. It was determined that a quadratic curve fit the standards and the standard curve was calculated for each assay test. The amount of Trolox Equivalents for each vegetable treatment was calculated from the average of the sample wells as μmol TE/g of vegetable on a fresh weight basis.

3.6 Texture Analysis

Texture analyses were performed on the cooked vegetables collected from the initial four replications. Texture was measure on a TA.XT texture analyzer, manufactured by Texture Technologies Company (Scarsdale NY). The texture of the broccoli was taken at the approximated center of the stem by placing the broccoli on a TA.90A flat plate and using a single probe (2 mm). Six measurements were taken from six different pieces of broccoli for each replication. Texture measurements were taken for the carrots by placing two carrot disks stacked on the flat plate and the 2 mm single probe was employed with six set of carrots. The peas and corn texture measurements were taken using a TA-52 2 mm probe French fry tester. Ten pieces of each vegetable were tested simultaneously for each replication.

3.7 Color Analysis

Hunter *L*,a*,b** system color analyses were performed using a Hunter Associates Laboratory (Reston, VA), Incorporated Miniscan XE Plus colorimeter after each of the first four replications. Color analyses for the thermally treated vegetables were performed upon cooling the vegetables in an ice-water bath to approximately 25^{o}C following each of the initial four heat treatments. Uncooked color analyses were performed by allowing frozen samples to thaw in a cooler overnight. The following morning three color measurements were taken with each vegetable. Broccoli was tested by cutting off the stems and placing florets in a 10 mm water activity cup (Aqua Lab, Pullman, WI) until the cup was filled with broccoli. Carrots were cut into pieces and used to completely fill the bottom of a cup. A second layer of carrots was placed onto the initial layer of carrots. The cups were filled with corn or peas. The lid of the cup was placed on top of the vegetable and pressure was applied until a flat surface of the vegetable was achieved that was level with the top of the cup.

3.8 pH Analyses

Analyses were conducted to determine the pH of the tap water used as the cooking medium and the pH of the vegetables following each of the two thermal treatments. The pH meter (Mettler Toledo DL12 Titrator, Hightstown, NJ) was calibrated prior to use each day. Samples of tap water were drawn on various days providing an average pH of the tap water available in the Pilot plant at The University of Tennessee. Frozen cooked samples were placed in a cooler and allowed to thaw overnight prior to pH analyses. The vegetables were transferred to a 250 mL plastic beaker and 50 mL of deionized water were added. A small hand-held blender was

employed to homogenize the vegetables and deionized water. The pH of the samples was then determined and recorded to 2 decimal places.

3.9 Statistical Analysis

Weights of the vegetable samples were determined before and after freeze drying to allow for determination of the moisture contents. The moisture content was used to convert the ORAC contents to a wet-weight basis since this is the form the vegetables are consumed. All analyses of ORAC values was on a wet-weight basis

Analysis of variance in Statistical Analysis Software (SAS) 9.1 using a randomized block design by blocking on the replications was used to perform the statistical analyses for each vegetable separately for ORAC contents and texture, color (L^*, a^*, b^*) and pH . Additionally, the ORAC content of the various treatments and vegetables was analyzed with SAS and blocked on each vegetable and replication. The SAS diagnostic output from each individual vegetable allowed for the identification of potential outliers. Normality of the data were also confirmed by SAS.

Microsoft® Excel software was used to determine averages and standard deviations for the ORAC and pH data for the 5 replications for each vegetable and treatment. Texture and color data averages and standard deviations were also determined by Microsoft® Excel for the 4 replications for each vegetable and treatment. Outliers were removed in the raw data, and all data reported are from the SAS runs with the outliers removed.

All treatment differences were determined by analysis of variance and at a significance level of $p<0.10$. SAS was used to determine correlations between vegetable color and ORAC assay values and between vegetable pH and ORAC assay values.

4. Results and Discussion

4.1 Oxygen Radical Absorbance Capacity Data

Appendix A contains ORAC data in a tabular format and Appendix F contains the weights of all vegetables prior to cooking and following cooking. Additionally, Appendix F contains the weights of all vegetables prior to and following freeze drying.

4.1.1 Vegetable Effects

The quantities of hydrophilic antioxidants present in the four vegetables as measured by ORAC were significantly different. Broccoli and corn were not statistically different ($p>0.10$) with overall average antioxidant amounts of 8.36 μmol TE/g and 6.45 μmol TE/g, respectively. The antioxidant capacity of peas was 5.47 μmol TE/g which was statistically significantly lower than broccoli but not corn. Carrot antioxidant capacity of 3.10 μmol TE/g was statistically lower than the antioxidant content in all other vegetables (Wu and others 2004).

4.2.1 Treatment Effects

Treatment heating effects also resulted in significant differences. The average amount of antioxidants present in all four uncooked frozen vegetables was 7.72 μmol TE/g. The average amount of antioxidants measured in the microwaved vegetables, 6.19 μmol TE/g, was not significantly different from the frozen vegetables but the average antioxidant content in the boiled vegetables, 3.64 μmol TE/g, was significantly lower ($p<0.10$) than the amounts in the frozen and microwaved vegetables.

4.2.2 Broccoli

The amounts of hydrophilic antioxidants present in three cooking treatments of

broccoli were statistically different ($p=0.074$) with 11.33 μmol TE/g for the uncooked sample, 8.04 μmol TE/g for the microwaved sample, and 5.72 μmol TE/g for the boiled sample. The microwaved sample was not statistically different than the uncooked or boiled sample, but the uncooked and boiled samples were different from each other. The retained water samples from boiled broccoli were also analyzed with the ORAC analysis, and these samples had a higher average ORAC value than the average ORAC value for the retained water samples from all other vegetables.

The heating and cooking in a small amount of water in the microwave may slightly affect the antioxidant content but not a statistically significant change from the boiled samples' antioxidant content as indicated in this research and other research (Turkmen and others 2005).

Other researchers determined antioxidant contents of uncooked broccoli samples were higher than in cooked samples (Wu and others 2004; Zhang and Hamauzu 2004). Wu and others (2004) found a 13.6 % decrease in the hydrophilic antioxidant capacity of boiled broccoli from uncooked broccoli employing the ORAC assay. Additional research, employing a different assay, determined the antioxidant capacity of boiled broccoli to decrease by approximately 65.0 % (Zhang and Hamauzu 2004). This research found a decrease in antioxidant capacity of approximately 49.4 % in boiled broccoli. The other researchers used fresh broccoli for their research; however, frozen broccoli was used for the current research. The antioxidant content likely decreases some due to blanching prior to freezing.

Boiling may have resulted in decreased levels of antioxidants present in the broccoli due to the boiling water causing the vegetable cells to rupture and antioxidants,

present in the cells, to leach from the cells into the boiling water (Price and others 1998). Thus, a significant decrease occurred in the boiled broccoli as compared to the uncooked broccoli.

4.2.3 Carrots

The amounts of hydrophilic antioxidants present in uncooked, microwaved, and boiled carrots were not statistically significant ($p=0.29$) and were 2.95 μmol TE/g, 4.00 μmol TE/g, and 2.39 μmol TE/g respectively. The retained water from boiled carrots had the lowest average ORAC value compared to the retained water ORAC values from all other vegetables. Carrots are considered an excellent source of carotenoids (Hager and Howard 2006). However carotenoids are fat-soluble; therefore, they are not part of the water-soluble extract analyzed for ORAC content. According to the literature carrots are comprised of approximately 66% to 95% hydrophilic antioxidants (Hunter and Fletcher 2002; Wu and others 2004). Since uncooked carrots did contain 2.95 μmol TE/g antioxidant content, no statistically significant difference in cooking methods indicated these antioxidants were possibly well protected at the cellular level (Hager and Howard 2006). Other research found the antioxidant content of boiled carrots was significantly lower than raw carrots (Wu and others 2004). A significant difference more likely occurs when comparing raw carrots to boiled carrots as opposed to frozen carrots to boiled carrots since the antioxidant content decreases during the blanching process. For example, raw carrots' hydrophilic antioxidant content was 11.6 μmol TE/g (Wu and others 2004). The raw carrots were cooked for approximately three min, in a manner similar to blanching, and their antioxidant capacity was determined to be 3.6 μmol TE/g (Wu and others 2004). The antioxidant content was 2.95 μmol TE/g for frozen uncooked

carrots in the current research. Therefore, the amount of antioxidants present in frozen carrots was comparable to the amount of antioxidants present following a blanching treatment. The blanching treatment for carrots would typically be longer than for some other vegetables to not only control enzyme changes but also to soften the texture of the frozen carrots.

4.2.4 Corn

Microwaving frozen corn resulted in a significantly larger quantity of antioxidants than boiled frozen corn with values of 8.12 μmol TE/g and 4.45 μmol TE/g respectively ($p=0.024$). The antioxidant content in uncooked frozen corn (6.32 μmol TE/g) was not statistically different than the amount of antioxidants found in either the boiled or microwaved corn ($p<0.05$). Microwaving has been reported to allow for greater antioxidant capacity in other vegetables, such as broccoli and squash (Turkmen and others 2005). The microwave cooking treatment possibly damaged the cell walls; thus allowing for easier extraction of antioxidants. Also, the possibility exists that antioxidants are formed during the microwaving process (Turkmen and others 2005). The loss of antioxidants due to boiling of corn most likely resulted from water-soluble antioxidants leaching into the water during cooking. Also, moisture gain occurred during boiling as seen in Table F2; therefore, the antioxidants remaining in the corn were diluted by the additional moisture.

4.2.5 Peas

The uncooked peas possessed significantly greater hydrophilic antioxidant content with a value of 10.2 μmol TE/g ($p=0.024$) than either microwaved peas with 5.14 μmol

TE/g or boiled peas with 2.43 μmol TE/g. The microwaving and boiling treatments were not statistically different. A greater variation existed among ORAC determinations in peas than the other vegetables; therefore, significant mean separations were less likely to occur. The cooked peas had lower antioxidant capacities than the uncooked peas possibly due to thermal processing rupturing cells thus allowing for hydrophilic antioxidants to leach into the cooking medium. Leaching may result in a loss of hydrophilic antioxidants during cooking. Research by Hunter and Fletcher (2002) determined frozen vegetables to have similar antioxidant contents as raw vegetables. Additionally, their research determined that 34.1% of the antioxidant activity was lost by boiling peas (Hunter and Fletcher 2002). The current research found 76.2 % of the hydrophilic antioxidant capacity was lost. The large difference in the decreased amounts of antioxidants was possibly due to different extraction methods and assays employed. Additionally, a longer cooking time was employed for the current research; therefore, a greater reduction was expected. Moisture gain occurred in the peas as it did in corn during boiling as seen in Table F2; therefore, the antioxidants remaining in the peas were diluted by the additional moisture.

4.3 Texture Analyses Data

Texture data is available in Appendix B in a tabular format.

4.3.1 Broccoli

The average texture analyses value for boiled broccoli was 1.31 N. The average microwaved broccoli's texture analyses value was 1.64 N. Statistical analysis by analysis of variance (ANOVA) found the textures to significantly vary at p=0.056. Boiled broccoli's texture was softer than the texture of the microwaved broccoli and both were

softer than the uncooked (thawed) control sample. The fact that the broccoli was not softened as much by microwaving did not result in an increased antioxidant content compared to boiled broccoli.

4.3.2 Carrots

The average texture analyses value for boiled carrots was 1.52 N. The average texture analyses value of the microwaved carrots was 2.68 N. A significant statistical difference at p=0.020 resulted from the varying cooking methods with regard to texture. As expected, the boiled carrots texture was softer and both cooked samples were softer than the uncooked (thawed) control sample. Again, the softer texture in the boiled vegetable did not result in significant effects on antioxidant content.

4.3.3 Corn

The average texture analyses value for boiled corn was 17.00 N. The average microwaved corn texture was 24.8 N. The texture of the boiled and microwaved corn did not vary significantly (p=0.507) and these samples were not different than the uncooked (thawed) control sample.

4.3.4 Peas

Boiled peas' average texture analyses value was 8.52 N. Microwaved peas' average texture analyses value was 8.25 N. Boiling and microwaving resulted in no significant differences as p=0.588 but they were softer than uncooked (thawed) control sample.

4.3.5 Texture overall

Corn and peas when heated in the microwave for 5 min. compared to boiled for

10 min. were similar in texture since they are composed of starch in the interior and primarily needed to be heated to a proper temperature rather than to a specific texture. The broccoli and carrots contain more fibrous materials, such as cellulose, and the boiling water is more effective in softening these fibers.

4.4 Color Analyses Data

Chlorophylls are the pigments that provide green color in foods. They are known to be sensitive to heat and pH. The orange, yellow, and red hues in foods are provided by carotenoids. Carotenoids are relatively stable to heat; therefore, the green colors of broccoli and peas are expected to alter more than the orange color of carrots and the yellow color of corn. The tables in Appendix C display color values.

4.4.1 Broccoli

The average *L*, a*, b** values for uncooked broccoli were: 35.73, -12.70, and 26.15 respectively. The average *L*, a*, b** values were: 42.20, -8.72, and 28.45 respectively for boiled broccoli and 39.68, -10.65, and 27.79 respectively for microwaved broccoli. Significant differences ($p<0.1$) were not found for the *b** value for uncooked, microwaved, and boiled broccoli among the replications. However, a significant difference was found for the *L** value for broccoli between the boiled value and the uncooked value. The broccoli became lighter following boiling which was possibly due to darker-colored pigments leaching into the water. The change in color was likely due to the sensitivity of the chlorophylls to heat. The *a** value for broccoli significantly changed with each of the cooking treatments. Uncooked broccoli had an *a** value of -12.7 whereas microwaved and boiled broccoli had *a** values of -10.6 and -8.72

respectively. The *a** value for broccoli indicated the broccoli became less green with microwaving and boiling. This is likely due to some of the chlorophyll being converted to pheophytin. The conversion to pheophytin occurred more extensively with boiling; therefore, the boiled broccoli was the least green. No significant correlations existed between broccoli color and antioxidant capacity ($p<0.1$).

4.4.2 Carrots

The uncooked average *L**, *a**, and *b** values were: 55.25, 37.71, and 46.55 respectively. Microwaved carrots average *L**, *a**, and *b** values were: 54.95, 35.93, and 54.63. The average *L**, *a**, and *b** values for boiled carrots were: 54.52, 35.68, and 52.93. The color analysis for carrots yielded no significant differences for either the *L** and *a** values for all of the treatments. This was expected as it is known that carotenoids are relatively heat stable pigments.

A significant difference was found for the *b** value at $p=0.0053$ for uncooked carrots and cooked carrots. Therefore, as the carrots were microwaved and boiled they became more yellow. The increase in yellow color was possibly due to chloroplasts and chromoplasts allowing for the leaching of carotenoids closer to the surface of the carrot. The color of carrots and the antioxidant capacity were not significantly correlated at $p<0.10$.

4.4.3 Corn

The uncooked average *L**, *a**, and *b** values for corn were: 76.71, 5.19, and 48.59 respectively. The average *L**, *a**, and *b** values for boiled corn were: 74.04, 8.77, and 54.98 respectively. The microwaved corn average *L**, *a**, and *b** values were: 71.62,

8.79, and 57.0 respectively. Significant differences were found for the *L**, *a**, and *b** values. The ends of the corn kernels were mechanically removed. The mechanical opening possibly allowed for starch to leach out of the corn during cooking; thus, partially accounting for the changes in the *L** and *b** values. The cooked corn samples, with a greater *a** value, were more red than the uncooked corn. This was possibly due to Maillard reaction end products which would be browner in color. The uncooked corn, which had a lesser *b** value, was less yellow. Research indicated carotenoids either are not affected by heat treatments or improve with heat treatments, such as canning (Scott and Eldridge 2004). Boiling and microwaving, while less severe heat treatments than canning, resulted in a more yellow color. Therefore, the current research was in agreement with previous findings. No significant correlations existed between the antioxidant capacity of corn and color at $p<0.1$.

4.4.4 Peas

The uncooked average *L**, *a**, and *b** values for peas were: 48.17, -17.83, and 39.43 respectively. The boiled peas average *L**, *a**, and *b** values were: 50.34, -15.46, and 37.74. The average *L**, *a**, and *b** values for microwaved peas were: 47.29, -15.82, and 36.58. The cooking methods yielded significant differences for the *L**, *a** and *b** values for the peas.

Chlorophylls are sensitive to heat; therefore, color changes were expected with peas. The uncooked peas were not significantly different than the peas cooked by both heating treatments; however, the microwaved peas were significantly darker than the boiled peas. Darker pigments possibly leached into the water during boiling accounting for the lighter color of the boiled peas as compared to the microwaved peas. The

uncooked peas, with the lesser a^* value, were greener than the cooked peas. The cooked peas were possibly less green due to chlorophyll conversion to pheophytin. The b^* value of the boiled peas was not significantly different than the uncooked and microwaved b^* values. However, the microwaved and uncooked b^* values were significantly different. The microwaved peas were less yellow than the uncooked peas. No significant correlations were found for the L^* and b^* value of peas and their antioxidant capacity. However, a significant correlation for the a^* value and color was determined for peas at p=0.046. The R value was 0.61, and the slope was negative. Therefore, the correlation between the a^* value and antioxidant capacity was a moderate, negative correlation. In other words, the antioxidant capacity was inversely proportional to the a^* value. Therefore, as the a^* value became smaller, resulting in a greener vegetable, the antioxidant capacity increased. The correlation between a greener vegetable and antioxidant capacity was expected as it is common knowledge that brightly colored foods are associated with greater quantities of antioxidants (Kalt 2005).

4.6 pH Analyses Data

The pH values for all vegetables are available in a tabular format in Appendix D.

4.6.1 Broccoli

The average pH value for boiled broccoli across the replications was 5.90, and the average pH value for microwaved broccoli was 6.22. The average pH value for the uncooked broccoli sample was 6.45. Statistically significant pH values were found in the boiled broccoli and the uncooked broccoli at p=0.103; however, the microwaved broccoli was not statistically different than the boiled broccoli or the uncooked broccoli. One possible explanation was that pectin or other compounds formed from acids were

degraded due to boiling; therefore, free acids were possibly formed. Formation of free acids provided a more acidic pH measurement. No correlation was found between pH and antioxidant capacity for broccoli ($p<0.1$).

FDA literature indicated the pH of frozen cooked broccoli ranged from 6.30 – 6.85 (FDA 2003). The cooking methods in this research indicated lower pH values than the FDA data. The FDA literature did not describe the cooking method employed to cook the broccoli. Differences in cooking methods possibly account for the lower pH values. The cultivar of the broccoli used in the FDA data was not provided. The cultivar in the current research possibly differed from the cultivar used in the FDA research; therefore, possibly differences in cultivars account for the varying pH values.

4.6.2 Carrots

The uncooked carrots average pH value was 6.69. The average pH value across all replications for carrots was 5.83 for boiled carrots and 5.89 for microwaved carrots. The uncooked carrots pH value significantly differed at $p=0.073$ than the pH values of the boiled carrots and microwaved carrots. The boiled and microwaved carrots' pH values did not statistically differ. The pH of carrots was not correlated to the antioxidant capacity at $p<0.1$.

Literature published by the FDA cited uncooked carrots' pH value ranging from 5.88 – 6.40 and cooked carrots' pH value ranging from 5.58-6.03 (FDA 2003). The pH value for the uncooked carrots was higher than the published pH value by the FDA for uncooked carrots. Possibly the difference in pH values is due to differing cultivars, varying growing conditions, or other inconsistent factors.

4.6.3 Corn

The average uncooked corn pH value was 7.34. The boiled and microwaved pH values for corn averaged across all replications were 6.49 and 6.92 respectively. Statistically at $p=0.0069$ the boiled corn and uncooked corn's pH values differed. However, the pH value of the microwaved corn did not statistically differ from either the boiled corn or the uncooked corn. The difference possibly resulted in free acids formed from the degradation of compounds comprised of amino acids due to boiling. No correlation between pH and antioxidant capacity existed for corn ($p<0.1$).

FDA literature cited a pH value of 6.40 for corn (FDA 2003). The same literature also stated the pH of canned corn ranging from 5.90 – 7.30. The 7.34 pH value for uncooked corn from this research was higher than the FDA pH value for uncooked corn. Again, different cultivars may have been tested. Additionally, the FDA data did not provide information on the number of samples tested to determine the reported pH value. Other factors possibly explain the difference in pH values, such as the growing climatic conditions of the corn, the growing location of the corn, and the type and amount of fertilizer used.

4.6.4 Peas

The average pH value of uncooked peas was 7.16. Boiled peas' average pH value across all Replications was 7.05, and the microwaved peas' average pH value across the Replications was 6.93. Statistically there were no significant differences among the cooking methods. However, the first Replication of peas was spoiled; therefore, it was eliminated from the pH analysis. Only having four replications of peas possibly accounted for the lack of treatment differences in pH values. Five replications were

tested for all other vegetables, and each of these vegetables experiences treatment differences in regard to their pH values. The pH of peas was not correlated ($p<0.1$) to the antioxidant capacity.

Published FDA literature for previously frozen cooked peas pH values ranged from 6.40 – 6.70. The reported values from this research are higher. Possible explanations include differing cultivars, varying growing conditions, and differing cooking methods. Also, the FDA data did not report the number of samples tested to determine pH which was possibly an additional source of variation.

5. Conclusions

Boiling as compared to microwaving or uncooked vegetables resulted in significantly lower quantities of antioxidants present in boiled vegetables, as determined by ORAC, for all vegetables across all replications. The uncooked (commercially frozen) vegetables served as the controls. The antioxidant contents of microwaved vegetables were not statistically different than the antioxidant contents of the control vegetables. Therefore as a general guideline, microwaving is considered the preferred heat treatment since it does not result in as great a loss of antioxidants as boiling does. Most of the antioxidants in the vegetables are hydrophilic antioxidants; therefore, it was expected boiling would cause a greater loss of antioxidants. The boiled vegetables were cooked in a greater quantity of water than the microwaved vegetables, and the boiling time was longer than the microwaving time. Thus, boiling provided more time for the water-soluble antioxidants in the vegetables to leach into the water. Additionally, moisture gain occurred during boiling for corn and peas; therefore, the antioxidants retained during boiling were diluted due to the additional moisture present in the vegetables.

The treatment effects for all vegetables were not followed for individual vegetables. Only boiled corn contained significantly less antioxidant capacity than microwaved corn. Boiled broccoli, carrots, and peas always contained the lowest measured antioxidant capacities compared to microwaved broccoli, carrots, and peas. However, these vegetables did not significantly vary in antioxidant capacity.

Other differences from the overall cooking treatment trend are that the antioxidant capacity of microwaved peas was significantly less than uncooked peas. Also, the antioxidant capacities of uncooked carrots and corn were not significantly different from antioxidant capacities of boiled carrots and corn.

Considering all treatments, broccoli and corn contained the largest antioxidant capacity. Peas had significantly less antioxidant capacity than broccoli, but the antioxidant capacity was not significantly different than in corn. The antioxidant capacity of carrots was significantly lower than all other analyzed vegetables.

The only significant correlation between color and antioxidant capacity was found for the a^* value of peas. The correlation was considered to be negative and of moderate strength. Therefore, as the peas became greener the antioxidant capacity increased.

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Appendices

Appendix A.

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Table A1 Hydrophilic ORAC values (µmol TE/g) ± SD for individual vegetables with varying heat treatments				
	Broccoli	**Carrots**	**Corn**	**Peas**
Uncooked	11.33 ± 2.23 A	2.95 ± 1.68 A	6.32 ± 2.18 AB	10.2 ± 5.71 A
Microwaved	8.04 ± 3.94 AB	4.00 ± 2.46 A	8.12 ± 1.39 A	5.14 ± 2.35 B
Boiled	5.72 ± 0.59 B	2.39 ± 1.68 A	4.45 ± 1.11 B	2.43 ± 1.08 B

Values are mean ± standard deviation

n = 5

mean ± standard deviation followed by a different letter are significantly different @ $p<0.10$ within each column

Table A2 Hydrophilic ORAC values (µmol TE/g) ± SD for heat treatments across all vegetables			
	ORAC Value	**Standard Deviation**	
Uncooked	7.69	± 4.47	A
Microwaved	6.20	± 3.22	A
Boiled	3.62	± 1.99	B

Values are mean ± standard deviation

n = 17

mean ± standard deviation followed by a different letter are significantly different @ $p<0.05$ within each column

Appendix B.

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Table B Texture values (N) ± SD for vegetables with varying heat treatments					
	Broccoli*	**Carrots***	**Corn°**	**Peas°**	
Uncooked	6.46	6.01	20.35	11.00	
Microwaved	1.64 ± 0.03	2.68 ± 0.39	24.8 ± 13.79	8.25 ± 0.74	
Boiled	1.31 N ± 0.21	1.52 ± 0.34	17.0 ± 1.32	8.52 ± 0.60	
p Value	0.06	0.02	0.33	0.59	

Values are mean ± standard deviation

n = 4

*single probe

° French fry tester

Appendix C.

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Table C1 Color Values (*L**) ±SD for Vegetables with varying heat treatments				
	Broccoli	**Carrots**	**Corn**	**Peas**
Uncooked	48.2 ± 1.3 A	55.3 ± 0.5 A	76.7 ± 0.7 A	48.2 ± 1.3 A
Microwaved	39.7 ± 1.2 A	55.0 ± 0.7 A	71.6 ± 1.7 C	47.3 ± 2.0 A
Boiled	42.2 ± 3.7 A	54.5 ± 0.5 A	74.0 ± 0.2 B	50.3 ± 0.9 A

Values are mean ± standard deviation

n = 4

mean ± standard deviation followed by a different letter are significantly different @ p<0.10 within each column

Table C2 Color Values (*a**) ± SD for Vegetables with varying heat treatments				
	Broccoli	**Carrots**	**Corn**	**Peas**
Uncooked	-12.7 ± 1.8 A	37.7 ± 1.0 A	5.2 ± 0.8 B	-17.8 ± 0.2 A
Microwaved	-10.7 ± 0.7 B	35.9 ± 1.2 A	8.8 ± 1.3 A	-15.8 ± 0.5 B
Boiled	-8.7 ± 0.6 B	35.7 ± 1.3 A	8.8 ± 0.6 A	-15.5 ± 0.6 B

Values are mean ± standard deviation

n = 4

mean ± standard deviation followed by a different letter are significantly different @ p<0.10 within each column

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Table C3 Color Values (*b**) ± SD for Vegetables with varying heat treatments				
	Broccoli	**Carrots**	**Corn**	**Peas**
Uncooked	26.2 ± 1.8 A	46.6 ± 1.0 B	48.6 ± 1.8 B	38.5 ± 1.8 A
Microwaved	27.8 ± 1.2 A	54.6 ± 1.3 A	57.0 ± 3.4 A	36.6 ± 0.9 C
Boiled	28.5 ± 1.4 A	52.9 ± 2.8 A	55.0 ± 1.9 A	37.7 ± 1.3 B

Values are mean ± standard deviation

n = 4

mean ± standard deviation followed by a different letter are significantly different @ $p<0.10$ within each column

Appendix D.

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Table D pH values ± SD for vegetables with varying heat treatments				
	Broccoli	**Carrots**	**Corn**	**Peas**
Uncooked	6.45 ± 0.23 A	6.69 ± 0.04 A	7.34 ± 0.04 A	7.16 ± 0.07 A
Microwaved	6.22 ± 0.43 AB	5.89 ± 0.63 AB	6.92 ± 0.47 AB	6.93 ± 0.31 A
Boiled	5.90 ± 0.56 B	5.83 ± 0.91 A	6.49 ± 0.51 B	7.05 ± 0.23 A

Values are mean ± standard deviation

n = 5 for the boiled and microwaved values all vegetables except peas, n = 4 for peas, and n = 3 for the uncooked values

mean ± standard deviation followed by a different letter are significantly different @ $p<0.10$ within each column

Appendix E.

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Table E1 Broccoli, frozen, chopped, unprepared nutrient information

Nutrient	Units	Value per 100 grams
Proximates		
Water		91.46
Energy	kcal	26
Protein	g	2.81
Total lipid (fat)	g	0.29
Ash	g	0.66
Carbohydrate, by difference	g	4.78
Fiber, total dietary	g	3
Sugars, total	g	1.32
Glucose (dextrose)	g	0.75
Fructose	g	0.83
Minerals		
Calcium, Ca	mg	56
Iron, Fe	mg	0.81
Magnesium, Mg	mg	18
Phosphorus, P	mg	50
Potassium, K	mg	212
Sodium, Na	mg	24
Zinc, Zn	mg	0.48
Copper, Cu	mg	0.038
Manganese, Mn	mg	0.294
Selenium, Se	mcg	2.8

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Table E1 con't., Broccoli, frozen, chopped, unprepared nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	56.4
Thiamin	mg	0.053
Riboflavin	mg	0.096
Niacin	mg	0.47
Pantothenic acid	mg	0.279
Vitamin B-6	mg	0.13
Folate, total	mcg	67
Folic acid	mcg	0
Folate, food	mcg	67
Folate, DFE	mcg_DFE	67
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	1029
Vitamin A, RAE	mcg_RAE	51
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	1.22
Vitamin E, added	mg	0
Tocopherol, beta	mg	0
Tocopherol, gamma	mg	0.27
Tocopherol, delta	mg	0
Vitamin K (phylloquinone)	mcg	91.6

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Table E1 con't., Broccoli, frozen, chopped, unprepared nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	610
Carotene, alpha	mcg	14
Cryptoxanthin, beta	mcg	1
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	1378

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Table E2 Broccoli, frozen, chopped, cooked, boiled, drained, without salt nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Proximates		
Water		90.72
Energy	kcal	28
Protein	g	3.1
Total lipid (fat)	g	0.12
Ash	g	0.71
Carbohydrate, by difference	g	5.35
Fiber, total dietary	g	3
Sugars, total	g	1.44
Glucose (dextrose)	g	
Fructose	g	33
Minerals		0.61
Calcium, Ca	mg	13
Iron, Fe	mg	49
Magnesium, Mg	mg	142
Phosphorus, P	mg	11
Potassium, K	mg	0.28
Sodium, Na	mg	0.034
Zinc, Zn	mg	0.223
Copper, Cu	mg	0.7
Manganese, Mn	mg	90.72
Selenium, Se	mcg	28

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Table E2 con't., Broccoli, frozen, chopped, cooked, boiled, drained, without salt nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	40.1
Thiamin	mg	0.055
Riboflavin	mg	0.081
Niacin	mg	0.458
Pantothenic acid	mg	0.274
Vitamin B-6	mg	0.13
Folate, total	mcg	56
Folic acid	mcg	0
Folate, food	mcg	56
Folate, DFE	mcg_DFE	56
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	1118
Vitamin A, RAE	mcg_RAE	56
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	1.32
Vitamin E, added	mg	0
Tocopherol, beta	mg	99.5
Tocopherol, gamma	mg	40.1
Tocopherol, delta	mg	0.055
Vitamin K (phylloquinone)	mcg	0.081

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Table E2 con't., Broccoli, frozen, chopped, cooked, boiled, drained, without salt nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	663
Carotene, alpha	mcg	15
Cryptoxanthin, beta	mcg	1
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	1498

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Table E3. Carrots, frozen, unprepared nutrient information

Nutrient	Units	Value per 100 grams
Proximates		
Water		90.04
Energy	kcal	36
Protein	g	0.78
Total lipid (fat)	g	0.46
Ash	g	0.83
Carbohydrate, by difference	g	7.9
Fiber, total dietary	g	3.3
Sugars, total	g	4.76
Glucose (dextrose)	g	4.05
Fructose	g	0.4
Starch	g	0.26
Minerals		
Calcium, Ca	mg	36
Iron, Fe	mg	0.44
Magnesium, Mg	mg	12
Phosphorus, P	mg	33
Potassium, K	mg	235
Sodium, Na	mg	68
Zinc, Zn	mg	0.33
Copper, Cu	mg	0.074
Manganese, Mn	mg	0.171
Selenium, Se	mcg	0.7

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Table E3 con't., Carrots, frozen, unprepared nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	2.5
Thiamin	mg	0.044
Riboflavin	mg	0.037
Niacin	mg	0.464
Pantothenic acid	mg	0.187
Vitamin B-6	mg	0.095
Folate, total	mcg	10
Folic acid	mcg	0
Folate, food	mcg	10
Folate, DFE	mcg_DFE	10
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	11242
Vitamin A, RAE	mcg_RAE	562
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.72
Vitamin E, added	mg	0
Vitamin K (phylloquinone)	mcg	17.6

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Table E3 con't., Carrots, frozen, unprepared nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	5300
Carotene, alpha	mcg	2890
Cryptoxanthin, beta	mcg	0
Lycopene	mcg	2
Lutein + zeaxanthin	mcg	298

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Table E4 Carrots, frozen, cooked, boiled, drained, without salt nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Proximates		
Water		90.32
Energy	kcal	37
Protein	g	0.58
Total lipid (fat)	g	0.68
Ash	g	0.69
Carbohydrate, by difference	g	7.73
Fiber, total dietary	g	3.3
Sugars, total	g	4.08
Sucrose	g	3.44
Glucose (dextrose)	g	0.36
Fructose	g	0.28
Starch		0.31
Minerals		
Calcium, Ca	mg	35
Iron, Fe	mg	0.53
Magnesium, Mg	mg	11
Phosphorus, P	mg	31
Potassium, K	mg	192
Sodium, Na	mg	59
Zinc, Zn	mg	0.35
Copper, Cu	mg	0.082
Manganese, Mn	mg	0.167
Selenium, Se	mcg	0.6

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Table E4 con't., Carrots, frozen, cooked, boiled, drained, without salt nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	2.3
Thiamin	mg	0.03
Riboflavin	mg	0.037
Niacin	mg	0.416
Pantothenic acid	mg	0.174
Vitamin B-6	mg	0.084
Folate, total	mcg	11
Folic acid	mcg	0
Folate, food	mcg	11
Folate, DFE	mcg_DFE	11
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	16626
Vitamin A, RAE	mcg_RAE	831
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	1.01
Vitamin E, added	mg	0
Vitamin K (phylloquinone)	mcg	13.6

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Table E4 con't., Carrots, frozen, cooked, boiled, drained, without salt nutrient information		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	8088
Carotene, alpha	mcg	3775
Cryptoxanthin, beta	mcg	0
Lycopene	mcg	2
Lutein + zeaxanthin	mcg	289

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Table E5 Corn, sweet, yellow, frozen, kernels cut off cob, unprepared

Nutrient	Units	Value per 100 grams
Proximates		
Water		74.92
Energy	kcal	88
Protein	g	3.02
Total lipid (fat)	g	0.77
Ash	g	0.48
Carbohydrate, by difference	g	20.81
Fiber, total dietary	g	2.4
Sugars, total	g	3.36
Sucrose	g	2.14
Glucose (dextrose)	g	0.52
Fructose	g	0.5
Maltose	g	0.19
Minerals		
Calcium, Ca	mg	4
Iron, Fe	mg	0.42
Magnesium, Mg	mg	18
Phosphorus, P	mg	69
Potassium, K	mg	210
Sodium, Na	mg	3
Zinc, Zn	mg	0.37
Copper, Cu	mg	0.036
Manganese, Mn	mg	0.126
Fluoride, F	mcg	14.6
Selenium, Se	mcg	0.7

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Table E5 con't., Corn, sweet, yellow, frozen, kernels cut off cob, unprepared		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	6.4
Thiamin	mg	0.083
Riboflavin	mg	0.07
Niacin	mg	1.726
Pantothenic acid	mg	0.28
Vitamin B-6	mg	0.178
Folate, total	mcg	36
Folic acid	mcg	0
Folate, food	mcg	36
Folate, DFE	mcg_DFE	36
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	217
Vitamin A, RAE	mcg_RAE	11
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.08
Vitamin E, added	mg	0
Tocopherol, beta	mg	0
Tocopherol, gamma	mg	0.15
Tocopherol, delta	mg	0
Vitamin K (phylloquinone)	mcg	0.3

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Table E5 con't., Corn, sweet, yellow, frozen, kernels cut off cob, unprepared		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	54
Carotene, alpha	mcg	19
Cryptoxanthin, beta	mcg	133
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	797

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Table E6 Corn, sweet, yellow, frozen, kernels, cut off cob, boiled, drained, with salt		
Nutrient	**Units**	**Value per 100 grams**
Proximates		
Water		77.03
Energy	kcal	79
Protein	g	2.55
Total lipid (fat)	g	0.67
Ash	g	1.03
Carbohydrate, by difference	g	18.71
Fiber, total dietary	g	2.4
Sugars, total	g	3.07
Sucrose	g	1.96
Glucose (dextrose)	g	0.48
Fructose	g	0.46
Maltose	g	0.17
Minerals		
Calcium, Ca	mg	3
Iron, Fe	mg	0.47
Magnesium, Mg	mg	28
Phosphorus, P	mg	79
Potassium, K	mg	233
Sodium, Na	mg	245
Zinc, Zn	mg	0.63
Copper, Cu	mg	0.048
Manganese, Mn	mg	0.155
Selenium, Se	mcg	0.7

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Table E6 con't., Corn, sweet, yellow, frozen, kernels, cut off cob, boiled, drained, with salt		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	3.5
Thiamin	mg	0.03
Riboflavin	mg	0.062
Niacin	mg	1.311
Pantothenic acid	mg	0.151
Vitamin B-6	mg	0.099
Folate, total	mcg	35
Folic acid	mcg	0
Folate, food	mcg	35
Folate, DFE	mcg_DFE	35
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	199
Vitamin A, RAE	mcg_RAE	10
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.07
Vitamin E, added	mg	0
Tocopherol, beta	mg	0
Tocopherol, gamma	mg	0.14
Tocopherol, delta	mg	0
Vitamin K (phylloquinone)	mcg	0.3

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Table E6 con't,, Corn, sweet, yellow, frozen, kernels, cut off cob, boiled, drained, with salt		
Nutrient	**Units**	**Value per 100 grams**
Other		
Carotene, beta	mcg	50
Carotene, alpha	mcg	17
Cryptoxanthin, beta	mcg	122
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	730

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Table E7 Peas, green, frozen, unprepared

Nutrient	Units	Value per 100 grams
Proximates		
Water		79.93
Energy	kcal	77
Protein	g	5.21
Total lipid (fat)	g	0.37
Ash	g	0.78
Carbohydrate, by difference	g	13.71
Fiber, total dietary	g	4.2
Sugars, total	g	5.38
Sucrose	g	4.74
Glucose (dextrose)	g	0.12
Fructose	g	0.37
Lactose	g	0.00
Maltose	g	0.16
Galactose	g	0.00
Minerals		
Calcium, Ca	mg	22
Iron, Fe	mg	1.53
Magnesium, Mg	mg	25
Phosphorus, P	mg	80
Potassium, K	mg	149
Sodium, Na	mg	112
Zinc, Zn	mg	0.81
Copper, Cu	mg	0.122
Manganese, Mn	mg	0.338
Selenium, Se	mcg	1.7

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Table E7 con't., Peas, green, frozen, unprepared		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	18
Thiamin	mg	0.258
Riboflavin	mg	0.1
Niacin	mg	1.707
Pantothenic acid	mg	0.136
Vitamin B-6	mg	0.122
Folate, total	mcg	53
Folic acid	mcg	0
Folate, food	mcg	53
Folate, DFE	mcg_DFE	53
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	2058
Vitamin A, RAE	mcg_RAE	103
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.03
Vitamin E, added	mg	0
Tocopherol, beta	mg	0
Tocopherol, gamma	mg	0.9
Tocopherol, delta	mg	0.02
Vitamin K (phylloquinone)	mcg	23.5

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Table E7 con't., Peas, green, frozen, unprepared		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	1225
Carotene, alpha	mcg	20
Cryptoxanthin, beta	mcg	0
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	2352

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Table E8 Peas, green, frozen, cooked, boiled, drained, without salt

Nutrient	Units	Value per 100 grams
Proximates		79.52
Water		78
Energy	kcal	327
Protein	g	5.15
Total lipid (fat)	g	0.27
Ash	g	0.8
Carbohydrate, by difference	g	14.26
Fiber, total dietary	g	5.5
Sugars, total	g	4.65
Sucrose	g	4.29
Glucose (dextrose)	g	0.12
Fructose	g	0.14
Lactose	g	0
Maltose	g	0.1
Galactose	g	0
Minerals		
Calcium, Ca	mg	24
Iron, Fe	mg	1.52
Magnesium, Mg	mg	22
Phosphorus, P	mg	77
Potassium, K	mg	110
Sodium, Na	mg	72
Zinc, Zn	mg	0.67
Copper, Cu	mg	0.105
Manganese, Mn	mg	0.279
Selenium, Se	mcg	1

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Table E8 con't., Peas, green, frozen, cooked, boiled, drained, without salt		
Nutrient	**Units**	**Value per 100 grams**
Vitamins		
Vitamin C, total ascorbic acid	mg	9.9
Thiamin	mg	0.283
Riboflavin	mg	0.1
Niacin	mg	1.48
Pantothenic acid	mg	0.142
Vitamin B-6	mg	0.113
Folate, total	mcg	59
Folic acid	mcg	0
Folate, food	mcg	59
Folate, DFE	mcg_DFE	59
Vitamin B-12	mcg	0
Vitamin B-12, added	mcg	0
Vitamin A, IU	IU	2100
Vitamin A, RAE	mcg_RAE	105
Retinol	mcg	0
Vitamin E (alpha-tocopherol)	mg	0.03
Vitamin E, added	mg	0
Tocopherol, beta	mg	0
Tocopherol, gamma	mg	2.47
Tocopherol, delta	mg	0.04
Vitamin K (phylloquinone)	mcg	24

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Table E8 con't., Peas, green, frozen, cooked, boiled, drained, without salt		
Nutrient	**Units**	**Value per 100 grams**
Other		
Alcohol, ethyl	g	0
Caffeine	mg	0
Theobromine	mg	0
Carotene, beta	mcg	1250
Carotene, alpha	mcg	20
Cryptoxanthin, beta	mcg	0
Lycopene	mcg	0
Lutein + zeaxanthin	mcg	2400

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Appendix F.

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Table F1 Average Microwaved Vegetable Weights*			
	$Veg_{prior\ to\ cooking}$ (g)	$Veg_{post\ cooking}$ (g)	% Loss
Broccoli	200.00	184.02	7.99
Carrots	200.00	180.52	9.74
Corn	200.00	184.26	7.87
Peas	200.00	187.18	6.41

***Note: All weight values are averaged for repetitions 1-5**

Table F2 Average Boiled Vegetable Weights*			
	$Veg_{prior\ to\ cooking}$ (g)	$Veg_{post\ cooking}$ (g)	% Loss**
Broccoli	200.00	201.72	-0.86
Carrots	200.00	193.36	3.32
Corn	200.00	212.18	-6.09
Peas	200.00	209.56	-4.78

***Note: All weight values are averaged for repetitions 1-5**

****** - indicates moisture gain**

Table F3 Average Freeze-dried Microwaved Vegetables Weights*		
	$Veg_{prior\ to\ freeze\ drying}$	$Veg_{post\ freeze\ drying}$
Broccoli	97.85	10.03
Carrots	99.23	10.65
Corn	103.28	31.75
Peas	101.05	24.55

***Note: All weight values are averaged for repetitions 2-5**

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Table F4 Average Freeze-dried Boiled Vegetables Weights*		
	$Veg_{prior\ to\ freeze\ drying}$	$Veg_{post\ freeze\ drying}$
Broccoli	100.13	8.13
Carrots	99.20	9.88
Corn	100.65	27.13
Peas	105.10	20.95

***Note: All weight values are averaged for repetitions 2-5**

Appendix G.

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Figure 1: Photographic image (cm) of frozen broccoli used in the research

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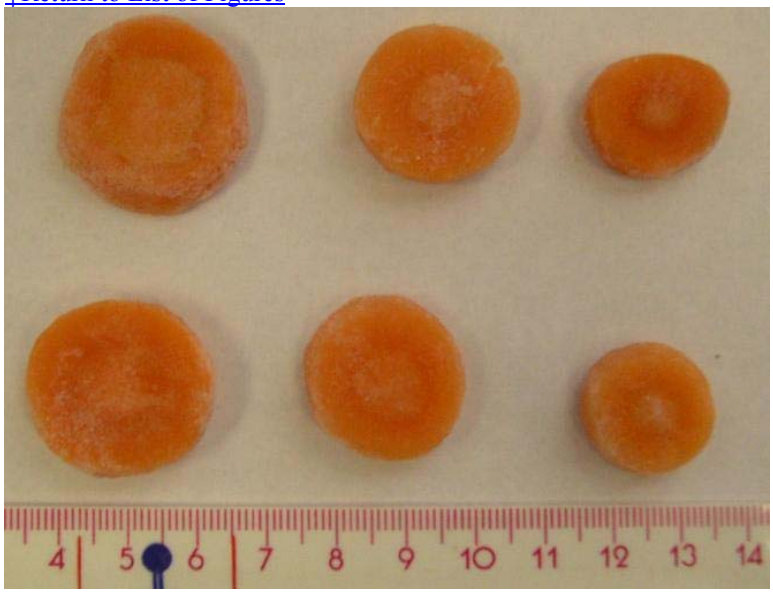

Figure 2: Photographic image (cm) of frozen carrots used in the research.

Appendix H.

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C1	B	B	B	B	B	B	B
B	S2	X3	X4	S3	X1	X2	B
B	X4	S4	X1	X2	S1	X3	B
B	S1	X2	X4	S2	X3	X1	B
B	X1	S3	X3	X2	S4	X4	B
B	B	B	B	B	B	B	B

Figure 3: Forty Eight Well Microplate employed for ORAC Assay for Replication 1

Where: C1, Fluorescein gain adjustment
B, Phosphate buffer working solution
S1, 3.125 microMolar Trolox working solution
S2, 6.25 microMolar Trolox working solution
S3, 12.5 microMolar Trolox working solution
S4, 25.0 microMolar Trolox working solution
X1, Sample 1 (varies with each ORAC assay performed)
X2, Sample 2 (varies with each ORAC assay performed)
X3, Sample 3 (varies with each ORAC assay performed)
X4, Sample 4 (varies with each ORAC assay performed)

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C1	B	B	B	B	B	B	B
B	S2	X3	X1	S3	X1	X2	B
B	X2	S4	X1	X2	S1	X3	B
B	S1	X2	X3	S2	X3	X1	B
B	X1	S3	X3	X2	S4	X3	B
B	B	B	B	B	B	B	B

Figure 4: Forty Eight Well Microplate employed for ORAC Assay for Replication 2 through Replication 5

Where: C1, Fluorescein gain adjustment

B, Phosphate buffer working solution

S1, 3.125 microMolar Trolox working solution

S2, 6.25 microMolar Trolox working solution
S3, 12.5 microMolar Trolox working solution
S4, 25.0 microMolar Trolox working solution
X1, Uncooked vegetable sample (varies with each assay performed)
X2, Microwaved vegetable sample (varies with each assay performed)
X3, Boiled vegetable sample (varies with each assay performed)

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C1	B	B	B	B	B	B	B
B	S2	X1	X2	S3	X3	X4	B
B	X5	S4	X6	X7	S1	X8	B
B	S1	X8	X7	S2	X6	X5	B
B	X4	S3	X3	X2	S4	X1	B
B	B	B	B	B	B	B	B

Figure 5: Forty Eight Well Microplate employed for initial ORAC Assay with retained water from boiled samples

Where: C1, Fluorescein gain adjustment

B, Phosphate buffer working solution

S1, 6.25 microMolar Trolox working solution

S2, 12.5 microMolar Trolox working solution
S3, 25.0 microMolar Trolox working solution
S4, 50.0 microMolar Trolox working solution
X1, Peas, Rep 1 at 1:100 dilution
X2, tap water, not diluted
X3, Peas, Rep 2 at 1:100 dilution
X4, Corn, Rep 1 at 1:100 dilution
X5, Carrots, Rep 1 at 1:100 dilution
X6, Corn, Rep 2 at 1:100 dilution
X7, Carrots, Rep 2 at 1:100 dilution
X8, Broccoli, Rep 2 at 1:100 dilution

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C1	B	B	B	B	B	B	B
B	S2	X1	X2	S3	X3	X4	B
B	X5	S4	X6	X7	S1	X8	B
B	S1	X8	X7	S2	X6	X5	B
B	X4	S3	X3	X2	S4	X1	B
B	B	B	B	B	B	B	B

Figure 6: Forty Eight Well Microplate employed for second ORAC Assay with retained water from boiled samples

Where: C1, Fluorescein gain adjustment

B, Phosphate buffer working solution

S1, 6.25 microMolar Trolox working solution

S2, 12.5 microMolar Trolox working solution
S3, 25.0 microMolar Trolox working solution
S4, 50.0 microMolar Trolox working solution
X1, Tap water at 1:100 dilution
X2, Peas, Rep 3 at 1:100 dilution
X3, Carrots, Rep 3 at 1:100 dilution
X4, Corn, Rep 4 at 1:100 dilution
X5, Carrots, Rep 4 at 1:100 dilution
X6, Broccoli, Rep 3 at 1:100 dilution
X7, Peas, Rep 4 at 1:100 dilution
X8, Corn, Rep 3 at 1:100 dilution

Appendix I.

1) Insert thermocouples in the second and third channels
2) Open the Chartview Plus 2.05.14 Program
3) Select RS-232
4) Select OK
5) Select “Upload Data During Acquisition” under Data
6) Ensure RS-232 is selected under interface which is under Device
7) Select Channels and Alarms under Setup
    a) Select the Channel and Alarm Setup tab
    b) Select on for “Ch 2” and “Ch 3” and ensure off is selected for all other channels
    c) Select Type “T” for “Ch 2” and “Ch 3”
    d) Select “^{o}C” for “Ch 2” and “Ch 3” units
8) Click on the Acquisition Setup tab
    a) Under Event Configuration select Channel Value for the Trigger
    b) Select Stop for Channel Value
    c) Select 2 for Channel
    d) Select 10 for Value
    e) Select 0 for Hyst
    f) Select the “Above” elevator button
9) Under Acquisition Parameters
    a) Select 1 Pre-trigger for scan counts
    b) Select 0 for Post stop
    c) Select 32 for average weight
    d) Select Normal for mode
    e) Select 0 for hours, 0 for minutes, and 1 for second under scan intervals and post trigger
    f) Select “use one interval”
10) Click on the Data Destination tab
    a) Provide a unique name for each experimental run
    b) Select the desired directory to store the data
11) Select “Arm Acquisition” under Acquire to begin the experimental run

Vita

The author graduated with a Bachelor's of Science degree in Chemical Engineering from The University of Tennessee in May 2003. Following graduation, she worked as a Chemical Engineer for BWXT Y-12, L.L.C. for two years. She entered graduated school at The University of Tennessee in August of 2005 to pursue a Master's degree in Food Science and Technology. She graduated with a Master's degree in 2007. She is a member of the American Institute of Chemical Engineers.